Null Results in Brief

No Association Between Glutathione Peroxidase Pro198Leu Polymorphism and Breast Cancer Risk

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Introduction

Selenium-dependent glutathione peroxidase (GPX1) is a cytosolic antioxidant enzyme that neutralizes H_2O_2 to water and oxygen (1). A *GPX1* Pro→Leu polymorphism exists at codon 198, with the variant Leu allele being less responsive than the common Pro allele to the stimulation of enzyme activity during selenium supplementation (2). The Leu allele has been associated with increased risk of lung (3), bladder (4), and breast (2) cancer, although two other studies found a null association with breast cancer risk (5, 6). Because oxidative stress may play a role in breast carcinogenesis (7) and the *GPX1* polymorphism may confer interindividual variability in the response to reactive oxygen species, we evaluated the association between the 198 *GPX1* polymorphism (RS#1050450) and risk of breast cancer, and assessed potential modifying influences of diet and lifestyle factors, which may affect reactive oxygen species, and tumor characteristics on risk relationships in the Long Island Breast Cancer Study Project.

Materials and Methods

The Long Island Breast Cancer Study Project, a population-based case-control study of breast cancer, was described previously (8). In brief, the cases were English-speaking women >20 years of age with newly diagnosed breast cancer who resided in Nassau and Suffolk Counties in Long Island, NY. Population-based controls were identified from the same geographic area, and frequency-matched to the expected age distribution of cases by 5-year age groups.

Known and suspected risk factors for breast cancer were ascertained by an in-person interview (8). Usual dietary intake was assessed by a self-administered modified National Cancer Institute–Block food frequency questionnaire (9). Genotyping was done by BioServe Biotechnologies (Laurel, MD) using Sequenom's high-throughput matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, as previously described (10), using PCR primers (5′-ACGTTGGATGATCCGAGCCTGACATCGAAGC-3′ and 5′-ACGTTGGATGATCCCGAGACAGCAGCA-3′).

There was excellent observer agreement in the 8% of randomly selected duplicates of genotyping results that were included for quality control purposes (κ statistic: 0.95), with <1% assay failure rate. Among those with DNA available (1,038 cases and 1,088 controls), 94% of cases and 93% of controls were Caucasian.

Unconditional logistic regression (11) was used to calculate odds ratios (OR) and corresponding 95% confidence intervals (CI) for breast cancer, in relation to genotype. The final multivariate models shown include matching factor (age) as well as those factors that changed the estimated effect by 10% or more (11). Factors found not to confound the associations of interest included: race, body mass index, age at first birth, smoking status, age at menarche, hormone replacement therapy use, menopausal status, benign breast disease, and lifetime alcohol intake. We examined potential interactions between *GPX1* genotypes and diet (fruit and vegetable consumption, and vitamin supplement; ref. 9), lifestyle factors (cigarette smoking, parity status, age at first birth, and lactation; refs. 8, 12), and tumor characteristics [*in situ* versus invasive, and estrogen receptor (ER)/progesterone receptor (PR) status; ref. 12]. Gene-environment interactions were evaluated by joint categories of *GPX1* genotype and diet and lifestyle factors. To test interactions on a multiplicative scale, a cross-product term of the ordinal score for each genotype and the specific risk factors was included in multivariate models. To test for potential heterogeneity by tumor characteristics, stratified analysis was done.

Results

Genotype distribution of *GPX1* followed Hardy-Weinberg equilibrium (P = 0.34) among controls. Genotype distribution and allele frequencies (Pro, 69%; Leu, 31%) were comparable with those observed in other published studies (2, 3, 5, 6). As shown in Table 1, having at least one *leu* allele (Pro/Leu and Leu/Leu genotypes) was not associated with breast cancer risk.

As shown in Table 2, there was little evidence for interaction between *GPX1* genotypes, selected breast cancer risk factors, and breast cancer risk in any of the models. In addition, there was little or no heterogeneity of risk with hormone receptor status. However, nulliparous women with variant Pro/Leu and Leu/Leu genotypes had increased risk [OR (95% CI) = 1.48 (0.99-2.23) and 2.12 (1.01-4.48), respectively], compared with parous women with common Pro/Pro genotypes, although cell sizes were small and risk estimates were somewhat unstable (P for multiplicative interaction = 0.21).

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Table 1. Breast cancer risk associated with *GPX1* polymorphisms (Long Island Breast Cancer Study Project, 1996-1997)

	Cases (%)	Controls (%)	OR* (95% CI)
Total participants	1,038 (100)	1,088 (100)	
Pro/Pro	472 (45)	523 (48)	1.00 (Ref)
Pro/Leu	456 (44)	453 (42)	1.10 (0.92-1.32)
Leu/Leu	110 (11)	112 (10)	1.06 (0.79-1.42)
Pro/Pro	472 (45)	523 (48)	1.00 (Ref)
Pro/Leu and Leu/Leu	566 (55)	565 (52)	1.09 (0.92-1.30)
Premenopausal women†	333 (100)	369 (100)	
Pro/Pro	155 (47)	177 (48)	1.00 (Ref)
Pro/Leu	134 (40)	158 (43)	0.97 (0.70-1.33)
Leu/Leu	44 (13)	34 (9)	1.44 (0.87-2.38)
Pro/Pro	155 (47)	177 (48)	1.00 (Ref)
Pro/Leu and Leu/Leu	178 (53)	192 (52)	1.05 (0.78-1.42)
Postmenopausal women†	693 (100)	664 (100)	
Pro/Pro	307 (44)	330 (50)	1.00 (Ref)
Pro/Leu	311 (45)	270 (41)	1.21 (0.96-1.52)
Leu/Leu	75 (11)	64 (10)	0.87 (0.60-1.25)
Pro/Pro	307 (44)	330 (50)	1.00 (Ref)
Pro/Leu and Leu/Leu	386 (56)	334 (51)	1.13 (0.91-1.41)

*Unconditional logistic regression adjusted for age.
†Excluding 67 subjects missing information on menopausal status.

Conclusions

Our data do not support the hypothesis that variant *GPX1* genotype is associated with an increased risk of breast cancer, confirming two previous studies. Knight et al. (6) reported that 198 *GPX1* polymorphism was not associated with breast cancer risk; however, a second *GPX1* allele containing four alanine repeats was associated with increased risk in same population. In the Nurses' Health Study, Cox et al. (5) found that both 198 Pro→Leu polymorphism and −1,040 G→A polymorphism (RS#3448) were not associated with the risk of breast cancer; significant linkage disequilibrium existed between them ($D' = 1.00$; $r = 0.4$; $P < 0.001$).

There are several possible explanations for the null association between *GPX1* genotypes and breast cancer. One possibility is that the effects of GPX1 on risk may only be observed in individuals with very high intake of selenium or fruits and vegetables, due to the observation that *in vitro* GPX1 enzyme activity differed between Pro and Leu alleles at high selenium supplementation (2). This is unlikely, however, because in our study, associations between *GPX1* genotype and breast cancer risk were null even among vitamin supplement users or higher fruit and vegetable consumers. Furthermore, mean consumption of total fruits and vegetables in Long Island Breast Cancer Study Project participants was higher than that of average women in the U.S. (26 svg/wk among National Health and Nutrition Examination Survey women versus 30 svg/wk among the Long Island Breast Cancer Study Project participants, excluding juice). Finally, our findings are based on a large population-based study, and we have adequate power (0.80) to be able to detect an OR of 1.28 or greater, with the sample size available.

We observed a somewhat suggestive interaction with parity status, although the exact mechanisms whereby *GXP1* effects may be greatest for nulliparous women need to be further investigated. Although our findings could be due to chance, it is also possible that women whose breast cells have never fully differentiated during a full-term pregnancy may be more susceptible to reduced capabilities for removal of reactive oxygen species by low-activity *GPX1* genotype, and thereby at increased risk of breast cancer.

In summary, we did not find evidence for associations between variant *GPX1* genotypes and breast cancer risk, nor

Table 2. Multivariate-adjusted ORs and 95%CIs for breast cancer in relation to *GPX1* polymorphisms, stratified by diet and lifestyle factors, and selected tumor characteristics (Long Island Breast Cancer Study Project, 1996-1997)

	Pro/Pro *GPX1*			Pro/Leu *GPX1*			Leu/Leu *GPX1*			*P* for multiplicative interaction
	Cases	Controls	OR (95% CI)*	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)	
Fruit and vegetables†										
0-22 svg/wk	164	169	1.00 (Ref)	144	144	1.01 (0.73-1.38)	37	35	1.08 (0.65-1.81)	0.67
22-37 svg/wk	164	169	0.96 (0.70-1.31)	166	156	1.05 (0.77-1.43)	34	42	0.77 (0.46-1.28)	
37+ svg/wk	137	177	0.73 (0.53-1.01)	138	146	0.89 (0.63-1.24)	36	34	0.96 (0.57-1.63)	
Vitamin supplement										
No	185	212	1.00 (Ref)	184	155	1.35 (1.01-1.81)	44	46	1.06 (0.67-1.68)	0.47
Yes	280	304	1.03 (0.80-1.33)	265	291	1.01 (0.78-1.31)	63	65	1.06 (0.71-1.59)	
Cigarette smoking										
Never	217	237	1.00 (Ref)	206	193	1.16 (0.88-1.52)	55	62	0.97 (0.64-1.47)	0.98
Former	155	178	0.94 (0.71-1.26)	167	178	0.98 (0.74-1.31)	39	35	1.09 (0.66-1.80)	
Current	100	107	1.15 (0.75-1.47)	83	81	1.21 (0.84-1.74)	16	15	1.13 (0.54-2.38)	
Parity status										
Parous	415	459	1 (Ref)	397	405	1.07 (0.88-1.29)	89	101	0.95 (0.70-1.31)	0.21
Nulliparous	57	64	1.05 (0.71-1.54)	59	48	1.48 (0.99-2.23)	21	11	2.12 (1.01-4.48)	
Age at first birth (among parous)										
Age FP <30 y	356	410	1.00 (Ref)	335	342	1.11 (0.90-1.36)	74	89	0.94 (0.67-1.32)	0.47
Age FP ≥30 y	59	49	1.49 (0.99-2.24)	62	63	1.20 (0.82-1.75)	15	12	1.48 (0.68-3.22)	
Lactation (among parous)										
Never	238	279	1.00 (Ref)	261	234	1.28 (1.00-1.64)	47	59	0.90 (0.59-1.37)	0.07
Ever	177	180	1.19 (0.90-1.56)	136	171	0.95 (0.72-1.27)	42	42	1.20 (0.76-1.91)	
Tumor‡										
In situ	75	523	1.00 (Ref)	79	453	1.22 (0.87-1.71)	25	112	1.56 (0.94-2.55)	
Invasive	397	523	1.00 (Ref)	377	453	1.08 (0.89-1.80)	85	112	0.97 (0.71-1.82)	
ER/PR status§,‡										
ER−/PR−	64	523	1.00 (Ref)	56	453	1.01 (0.69-1.48)	13	112	0.94 (0.50-1.78)	
ER+ or PR+	248	523	1.00 (Ref)	234	453	1.07 (0.86-1.88)	52	112	0.93 (0.64-1.84)	

*ORs and 95% CIs calculated by unconditional logistic regression, adjusted for age and total calorie (only for fruit and vegetable).
†Fruit and vegetable consumption based on tertiles of control group.
‡To test for potential heterogeneity by tumor characteristics, stratified analysis was done.
§Excluding 371 subjects missing information on ER/PR status.

was the association modified by diet or tumor characteristics in the Long Island Breast Cancer Study Project. However, we did find that risk was somewhat elevated among nulliparous women with the variant *GPX1* genotype, compared with parous women with the common *GPX1* genotype.

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